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Original Investigation

Effect of Cepea Extract-Heparin and Allantoin Mixture on Epidural Fibrosis in a Rat Hemilaminectomy Model

Cepea Ekstresi-Allantoin ve Heparinin Rat Hemilaminektomi Modelinde Oluşturulan Epidural Fibrozisi Önlemedeki Etkinliği

Cuneyt TEMIZ[1]
Peyker TEMIZ[2]
Murat SAYIN[3]
Kubilay UCAR[4]

[1,3] Celal Bayar University School of Medicine, Neurosurgery Department, Manisa, Turkey
[2] Celal Bayar University School of Medicine, Pathology Department, Manisa, Turkey
[4] İzmir Educational Hospital, Neurosurgery Clinic, İzmir, Turkey

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Correspondence address:
Cuneyt TEMIZ
E-mail: temiz2@tr.net

ABSTRACT

AIM: Epidural fibrosis following a laminectomy procedure is a serious problem that results in failed back surgery syndrome. A serious number of manuscripts have explained its possible mechanism and results but no effective preventive surgical technique or treatment is currently present.

MATERIAL and METHODS: We used a rat hemilaminectomy model at lumbar fourth level. In the treatment group (n:10), the hemilaminectomy sites were filled with cepea extract-allantoin and heparin mixture as sterile cream form. In the second group, the same surgical procedure was performed and the site was filled with physiological saline. All animals were terminated after 6 weeks and laminectomy sites removed en-bloc. Epidural fibrosis was evaluated and compared using semi-quantitative histopathological scoring scales.

RESULTS: In the physiological saline group, the fibrosis score was 10.3 points and 90% of the subjects had acute inflammatory reaction, 80% chronic inflammatory reaction and 100% showed bone destruction and reparation process. In the cepea extract group, these values were fibrosis score 4.2 points, 0% acute inflammatory reaction, 33.3% chronic inflammatory reaction and 10% bone destruction and reparation process, respectively.

CONCLUSION: This study showed that aloe cepea extract-allantoin and heparin mixture diminished epidural scarring formation effectively with decreased scores of acute and chronic inflammation, compared to the physiological saline solution group.

KEYWORDS: Epidural fibrosis, Cepea extract, Heparin, Allantoin

ÖZ

AMAÇ: Hemilaminektomi sonrası gelişen epidural fibrosis, başarısız bel cerrahisi sendromuna neden olabilen ciddi bir sorundur. Epidural fibrozisin muhtemel mekanizmalarıyla ilgili birçok yazı mevcut olmakla birlikte önleyici cerrahi teknik veya tedavi halen bulunamamıştır.

YÖNTEM VE GEREÇ: Dördüncü vertebra da rat hemilaminektomi modeli kullanıldı. Tedavi grubunda(n:10), laminektomi alanı steril cepea ekstresi, allantoin ve heparin karışımı ile dolduruldu. İkinci gruba da aynı cerrahi işlemler uygulandıktan sonra alan aynı hacimde serum fizyolojik ile dolduruldu. Altı haftalık takip sonrası hemilaminektomi alanları bir bütün olarak çıkarılarak histopatolojik olarak derecelendirildi.

BULGULAR: Tedavi grubunda fibrosis derecesi 4,2 iken serum fizyolojik grubunda 10,3 olarak tespit edildi.Serum fizyolojik grubunda örneklerin %90'nında akut enflamatuvar reaksiyon, %80'ninde kronik enflamatuvar reaksiyon ve örnekleri tamamında kemik yıkım ve yapım süreçleri gözlendi. Cepea ektre grubunda örneklerin hiçbirinde (%0) akut enflamatuvar reaksiyon,%33,3 kronik enflamatuvar reaksiyon ve %10'nunda kemik yıkım ve yapımı tespit edildi.

SONUÇ: Bu çalışma ile cepea ekstresi, allantoin ve heparin karışımının epidural fibrozisi serum fizyolojiğe kıyasla; akut ve kronik enflamasyonu puansal ve istatistiksel olarak ile anlamlı ($p<0.05$) biçimde azalttığı gösterilmiştir.

ANAHTAR SÖZCÜKLER: Epidural fibrozis, Cepea ekstresi, Heparin, Allantoin

INTRODUCTION

Epidural fibrosis after hemilaminectomy is a serious cause of morbidity after lumbar disc surgery (1,3,5). Insufficient attenuation or renewal of pain after surgery, known as failed back surgery syndrome, is one of the results of this procedure (16). New neurological deficits may also occur due to re-compression of the nerve root by scar tissue (9).

Some antineoplastic agents such as mitomycin C, 5-fluorouracil, cyclosporin (24) and some high molecular weight molecules such as hyaluronan (18), oxidized regenerated cellulose (22), free fat grafts (7), steroid solutions, dural adhesion barriers (13, 21, 25), various chemical agents (4,8,17,19,20,25), and many surgical techniques such as smooth dissection or preservation of ligamentum flavum (15), and even external beam radiation therapy (6) have been used to reduce or inhibit epidural fibrosis formation.

Free fat graft implantations and dura grafts or barrier gels had only little value on a practical basis (7, 21, 22). New surgical techniques have promised a better prognosis but they are far from a total solution for the problem.

In this study, we aimed to investigate the effect of cepea extract, allantoin and heparin mixture on epidural fibrosis formation on a rat lumbar hemilaminectomy model. Fibrosis formation was assessed and classified mainly by histopathological findings.

MATERIALS and METHODS

Surgical Technique:

This study has been approved by the animal research ethical committee of Celal Bayar University. We used 20 male Sprague-Dawley rats weighing between 250 to 300 grams. One hour prior to surgical intervention, cephazolin sodium (Iespor, Ulagay-İstanbul-Turkey, 2007) was injected via the intramuscular route (20 mg/kg). Rats were anesthesized with intraperitonally administrated ketamine hydrochloride (2 mg/kg) (Ketalar, Pfizer-Lüleburgaz-Turkey, 2007) and stabilised on the operation table in the prone position. Following sterile isolation, 3 centimeter=long midline surgical insicions were performed between the lumbar first and fifth levels. On the right side, paravertebral muscles were dissected in a subperiostal fashion and L4 laminae were exposed. Hemilaminectomies were performed using a high-speed electrical drill (Universal BM 24A, BM Motors-Istanbul-Turkey) and 1 mm. Kerrison rongeurs under 4X optical magnification (Möller-Wedel D 22880, Möller-Wedel Co., GmbH- Germany) until dura mater and epidural spaces were exposed. Minor bleeding was controlled with meticulous bipolar coagulation.

Drug Administration:

A total of 20 hemilaminectomies were obtained using this technique. 10 hemilaminectomy sites were filled with 1 cc. sterile cepea extract (100 mg/g), allantoin (10 mg/g) and heparin (50 IU/g) mixture in cream form (Contractubex, Merz Co., GmbH-Germany, 2007). Sterilization of cream was achieved in an autoclave under 2 ATM pressure at 160 °C temperature. 10 sites were filled with physiological saline solution (Eczacıbaşı- Baxter, Istanbul-Turkey, 2007) at the same volume. Incisions were closed with 3/0 vicryl in anatomical manner. The rats were then left for free food and water consumption for six weeks.

After six weeks, the rats were terminated with intraperitoneally administrated thiopental sodium (Pental, Ulagay,İstanbul-Turkey, 2007) solution (10 mg/kg). The fourth and fifth vertebrae were excised with their laminae, dural sacs, nerve roots and paravertebral tissues including muscles and skin.

Histopathological evaluation:

All specimens were put into 10% formalin solution for preparation for histopathological evaluation (n:10 for cepea extract and n:10 for physiological saline solution groups). Specimens were cut into 2 milimeter-thick axial slices and left for formalin fixation for 48 hours. Each tissue sample was decalcified with 10% nitric acid solution for 48 hours. All specimens were then washed with tap water for 12 hours. Five μm-thick serial sections were cut from formalin-fixed paraffin-embedded tissues and stained with hematoxylin and eosin.

The formalin-fixed paraffinized tissue sections contained whole tissue layers including skin, subcutaneous tissue, paravertebral muscles, bone and dura mater-nervous tissue. The inflammatory changes and scar tissue development were observed mostly in the epidural areas but reached subcutaneous tissues in some areas. Inflammation, scar tissue development and other histopathological changes were determined in the whole thickness of these tissue layers under 20-fold optical magnification

These sections were examined microscopically using the criteria below classifying inflammation and fibrosis. All microscopic examinations were performed

by the same investigator who was blinded during the evaluation. Inflammation/ fibrosis score and inflammation/ fibrosis grades were obtained according to the following criteria:

Histopathological classification:

Fibrosis levels were assessed using a four-level grading system;

0- No fibrotic tissue
1- Superficial or focal fibrosis
2- Superficial-spread or deep-local fibrotic tissue
3- Deep and spread fibrosis

Oedematous changes in tissues were assessed in two levels;

0- No oedematous changes
1- Oedematous changes

Acute inflammatory cell density levels were assessed in four levels;

0- No inflammatory cells
1- Focal and few cells
2- Spreading and many cells
3- Abscess formation

Chronic inflammatory cell density levels were assessed in three levels;

0- No inflammatory cells
1- Focal and few cells
2- Spreading and many cells

Chronic inflammatory granulation tissue levels were assessed in two levels;

0- Absent
1- Present

Bone destruction and healings were assessed in three levels;

0- No bone destruction
1- Enchondral ossification and fibrosis
2- Destructed bone, spicule formation, fibrosis and surrounding inflammation response

Total scores were calculated between 0 to 12 points according to this scale. 0 to 5 points was named degree 1 and resembled light inflammation and fibrosis, 6 to 8 points was named degree 2 and resembled moderate inflammation and fibrosis, 9 to 12 points was named degree 3 and resembled heavy inflammation and fibrosis.

Statistical analysis were made with the Mann-Whitney U test using the SPSS 14.0 for Microsoft Windows program and a p value <0.05 was considered statistically significant.

RESULTS

In physiological saline group, the mean inflammation-fibrosis score was 10.3 points (Table I). This means heavy inflammation and fibrosis. This value was at 4.2 points at treatment group and could be explained light inflammation and fibrosis as regards the histopathological grading system (Table II). All subjects' (100%) inflammation/ fibrosis grade was 3 in the physiological saline group and 1 in the treatment group. Total score and grade differences between the two groups were statistically significant ($p<0.05$) (Figure 1). These results indicate that cepea extract decreased epidural fibrosis and inflammation compared to physiological saline.

Further analysis of histopathological findings revealed that oedematous changes was seen in 90% of

Table I: Physiological saline group values.

Rat no	Fibrosis	Acute inflammation	Oedema	Chronic inflammation	Chronic inflammatory granulation tissue	Bone destruction, reparation	Total score	Degree
1	3	3	1	1	0	2	10	3
2	3	3	1	1	1	2	11	3
3	3	0	1	2	1	2	9	3
4	2	3	1	1	0	2	9	3
5	3	2	0	2	1	2	10	3
6	3	1	1	2	1	2	10	3
7	3	3	1	2	1	2	12	3
8	3	2	1	1	1	2	10	3
9	3	1	1	2	1	2	10	3
10	3	3	1	2	1	2	12	3

Mean: 10.3 p± 1.5 p.

Table II: Cepea extract group values.

Rat no	Fibrosis	Acute inflammation	Oedema	Chronic inflammation	Chronic inflammatory granulation tissue	Bone destruction, reparation	Total score	Degree
1	1	0	1	0	0	1	3	1
2	2	0	1	0	0	1	4	1
3	2	0	1	0	0	2	5	1
4	1	0	1	1	0	1	4	1
5	2	0	0	0	0	1	3	1
6	2	0	1	0	0	1	4	1
7	2	0	1	1	0	1	5	1
8	2	0	1	0	0	1	4	1
9	1	0	1	1	1	1	5	1
10	2	0	1	0	0	1	5	1

Mean: 4.2 p±1.5 p.

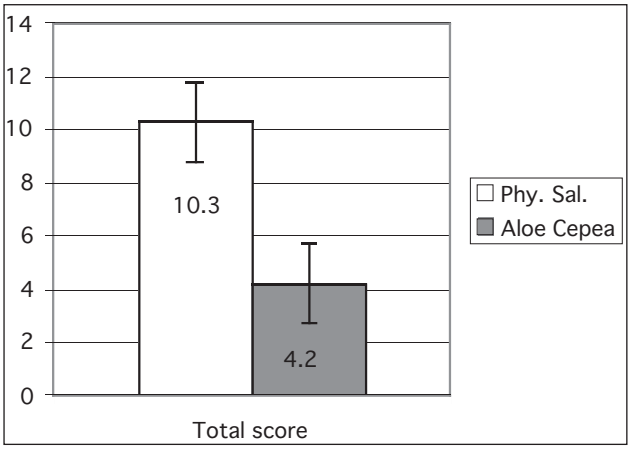

Figure 1: *Total scores of physiological saline and cepea extract groups (Mean±SD) Standard deviations were at 1.5 points for both groups.*

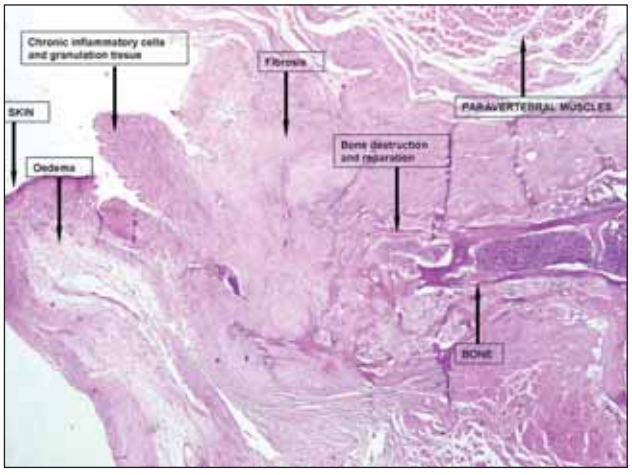

Figure 2: *Destructed bone and widespread new bone formation with marked fibrosis and surrounding inflammation response (acute and chronic inflammatory cells, granulation tissue and oedema) in control group (HEx20).*

the subjects in the cepea group and 90% of the subjects in physiological saline group and the difference was not statistically significant.

No acute inflammatory reaction was observed in the cepea extract applied group (Figure 3) but 90% (9/10) of the subjects had acute inflammation in the physiological saline group. Score 3 acute inflammation (abscess formation) was seen in 50%, score 2 (spreading and many cells) in 20% and score 1 (focal and few cells) in 20% of the subjects in the physiological saline group (Table I). There was a statistically significant difference between two groups for acute inflammation. ($p< 0.05$). It seems that cepea extract effectively diminished acute inflammatory reaction compared to physiological saline.

Chronic inflammatory cell infiltration was seen in 3 subjects (33.3%) in the cepea extract group and both were at score 1 (focal and few cells). In physiological saline group, chronic inflammatory cell infiltration was seen in 100 % (10/10) (the score was 1 in 40% and 2 with spreading and many cells in 60%). The difference was statistically significant ($p< 0.05$). Chronic inflammatory granulation tissue was seen in 80% of subjects in the physiological saline group and only one of the subjects (10%) in cepea group (Table II). There was also a statistically significant difference regarding chronic inflammatory granulation tissue ($p< 0.05$). These can be interpreted as cepea extract effectively inhibiting the chronic inflammatory process compared to physiological saline.

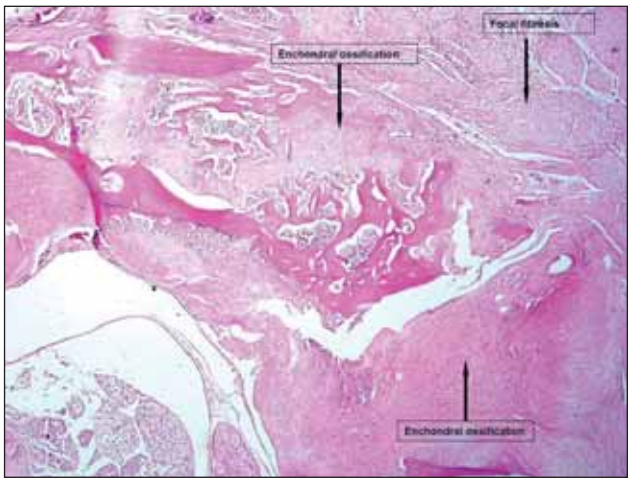

Figure 3. *A sample of treatment group showed mild enchondral ossification and focal fibrosis but no acute inflammation, oedema, chronic inflammatory cells and granulation tissue (HE, x20).*

Bone destruction and reparation findings score was 1 (enchondral ossification and fibrosis) in 90% (9/10) of subjects in the cepea group, and the score was 2 in only 1 subject (10%) (destructed bone, spicule formation, fibrosis and surrounding inflammation response), whereas all subjects (100%) were score 2 in the physiological saline group. The difference between two groups regarding bone destruction and reparation was statistically significant ($p<0.05$). These results showed that cepea extract also diminished bone destruction without inhibiting enchondral ossification.

DISCUSSION

Postoperative epidural fibrosis is a serious consequence following most spinal procedures (5). Extensive fibrous tissue formation is usually associated with morbidity (9). Morbidity mainly occurs as renewal of pain and even neurological deficits. Reports have suggested that postoperative epidural scar tissue is an aetiological factor in a significant proportion (up to 24%) of cases of failed back syndrome (11,16).

Many surgical procedures (15,20) and materials have been suggested as means of preventing or limiting the development of fibrosing tissue after lumbar discectomy. The first and most promising materials were free or pediculated fat grafts prepared from normal subcutaneous tissue (7,19). Unfortunately, these grafts showed little benefit because of histological differences between epidural and subcutaneous fat tissues. Numerous synthetic materials and chemical agents have also been used as ways to control epidural fibrosis and these include hyalurone (18), DuraGen (21), oxiplex (Fziomed Inc.) (10), and Adcon-L (Gliatech Inc.) (25). Although some reports suggested satisfactory outcomes with these materials others have not. It has also been demonstrated that application of steroids or non-steroidal anti-inflammatory agents can alleviate epidural fibrosis (11) and application of hemostatic agents can curb this process. None of these chemical agents or barrier materials showed high success rates in means of preventing epidural fibrosis formation in a practical manner.

While heparin-soaked collagen membrane is known to prevent fibrosis formation after laparoscopic procedures, a numerous number of reports have been published regarding its effect on posthemilaminectomy epidural fibrosis (12). Some studies reported a beneficial effect on preventing scar formation.

Allantoin is known to have supportive effects on the primary and secondary wound healing process (2, 14, 23). Aloe cepae extract is a natural molecule that is derived mainly from onion. The chemical formula is $C_4H_6N_4O_3$ and it is named 5- ureahydantoin or glyoxydiureide. It is a metabolite of purine oxidation and is mainly extracted by urine. This molecule has bactericidal and anti-inflammatory effects. Heparin has anti-inflammatory effects also and helps water binding to scar tissue. On the other hand, heparin helps to increase microcirculation.

Cepea extract, allantoin and heparin in a mixture together show increased effect and the result is increased diffusion capability into tissues (23). These effects of the mixture have been used successfully for many years for promoting the skin wound healing process without scar formation (2).

There are many reports at the literature suggesting the decreasing effect of this mixture on scar formation in various organs and systems in the human body.

Willital et al. reported that a mixture of cepea extract-allantoin and heparin diminished fresh scar tissue formation following thoracal surgery (23).

Maragakis stated that this mixture not only decreased fibrosis formation but also diminished dermal scar and keloid formation after thoracal surgery (14).

Beuth has shown that heparin-cepea extract and

allantoin mixture decreases dermal scar formation more effectively than steroids (2).

The local application of cepea extract- heparin and allantoin mixture diminished epidural scar formation in a statistically significant manner in this study. Our results showed that this mixture also decreased inflammatory reaction of tissue both in acute and chronic phases. This effect of this mixture could be produced both by inhibiting inflammatory cell migration and decreasing chronic inflammatory granulation tissue formation. Bone destruction was prominent and no reparation process could be seen in the control group. Interestingly, enchondral ossification and bone reparation continued in the treatment group. We can therefore suggest that bone reparation became better or healthier with this mixture. Best of all, cepea extract-heparin and allantoin mixture is cheap and can be sterilised and applied safely.

To the best of our knowledge, this is the first report with this level of histopathological evaluation of the diminishing effect of locally applied cepea extract, allantoin and heparin mixture on postoperative epidural fibrosis formation without depressing the bone reparation process after a hemilaminectomy procedure.

We believe that the effects of this mixture need further evaluation in future studies.

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